THE GROWTH OF AMŒBA ON A SOLID MEDIUM FOR CLASS USE

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In December, 1914 the class in laboratory methods at the University of Michigan was studying the methods of isolating and culturing for class use, various living forms, especially protozoans. The amœba proved to be the most difficult. It was not practical to isolate in order to start a pure culture, and those cultures which were made, were more or less uncertain, both as to the number of amœba and the time they would last. Dr. George R. La Rue, the instructor of the class, suggested that it might be possible to grow some forms for class use on a solid medium.

The preliminary work was just under way when Dr. James B. Pollock, in isolating Azotobacter from the soil, found a large number of small amœbæ in his cultures. These were examined and cultured on various media. Doctor Pollock pointed out that Azotobacter would be a good organism to use for feeding amœbæ, as this bacterium can be cultured on nitrogen-free media. Such media would not readily become contaminated with many fungi and bacteria which had been giving trouble in the previous work. This has been the essential key to all future work. A summary of the experiments on culturing the soil amœbæ was read before the Michigan Academy of Science, 1915 by Miss Kniseley, who had carried on the work as a class problem.

In May, 1915 the work of further culturing amœbæ was assigned to me, as Miss Kniseley had left the problem unfinished. On May 20th inoculations were made onto ten slants of Ashby's Agar Medium (Ashby, 1907: 54) from the inactive tubes of Miss Kniseley. In three to five days these all showed large numbers of very active soil amœbæ. In twelve to fourteen days practically all of the amœbæ had become encysted. All later observations have borne out these results. Further work has not been done on the life history of this amœba.

The object of my work was to find some form suitable for class study which associated with an easily attainable bacterium would grow on a synthetic medium. Many attempts were made to isolate large amœbæ from aqueous cultures and to inoculate the soil amœba cultures with them. None of these proved successful, and in June preparations were made to keep the cultures running over the summer. For this purpose a new medium was selected. This was suggested in a paper by Miss Mockridge (1912:871). The formula used was a modification of the formula given in her paper for growing Azotobacter. The medium as used is as follows:

Dextrine	10.0 grm.
Di-potassium phosphate	2.0 grm.
Magnesium sulphate	0.2 grm.
Calcium carbonate	0.2 grm.
Agar agar	10.0 grm.
Distilled water	1000.0 c. c.

It should be noted that this formula is for a nitrogen-free nutrient solution. This character makes it practically possible to inoculate from aqueous cultures without serious contamination.

During the summer the slants and cultures were taken to Lane College, Chicago and attempts to isolate large amœba and inoculate onto the cultures were continued. An aqueous culture obtained while there, proved to be very plentifully filled with amœbæ—five to ten in most low power fields. Attempts to isolate and inoculate from this culture proved unsuccessful. A rougher method was then tried. A few drops of the aqueous culture were put into each of ten of the tube cultures of Azotobacter and soil amœbæ. In twenty-four hours two of the tubes were plentifully covered with the larger amœbæ, and in three days all showed excellent growths. These ten tubes remained active for sixteen days to two months. Transplants from these cultures in an active state gave new growths of Azotobacter, soil amœbæ and large amœbæ. Various other forms that were in the original aqueous cultures have disappeared after numerous transplants. Now, after 270 transplants and selections, the cultures apparently show only bacteria, a tiny fungus, soil amœbæ, and the larger amœbæ.

This large amœba is very excellent for class use. When extended it measures from 90μ to 150μ. In general appearance it is very much like *Amœba proteus,* but it is slightly smaller, and its pseudopodia are frequently sharper than in the most typical form of *Amœba proteus.* It is readily mistaken for *Amœba proteus* until it is killed and stained, in which case the nucleus is found to be round with a clearly marked nuclear membrane. In the cultures the amœbæ are quite active, as well as being numerously distributed. One small platinum loop from the culture may have from 50 to 200 or more amœbæ. One tube will furnish sufficient material for a class of 40. These cultures have been used with good success this year at the University of Michigan and in various high schools.

Once started, it is very easy to keep cultures of this amœba going indefinitely, thus assuring plenty of amœbæ for class use at all times. The cultures are kept as any ordinary bacterial culture, no special methods being necessary. Transplants must be made while the amœbæ are growing, and preferably while they are numerously distributed over the culture. They may be kept at ordinary room temperature, but must not be exposed to direct sunlight. It is best to keep ten cultures going in order to insure a good quantity in some of the tubes. A life history of one of the tubes will show how often transplants have been made; these were usually made from the tubes having the most amœbæ present in them.

Date of original inoculation from aqueous cultures, 6/30/15.

Tube Nos.	Date of Inoculation	Source
II G.	5/10/16	II N
II N	4/5/16	II A
II A	3/8/16	II C
II C	2/15/16	II A
II A	12/17/15	Fr II_3
Fr II_3	11/30/15	Fr II_2
Fr II_2	9/21/15	Fr II

Two plans of procedure for cultivation of amœbæ are here given. The work should be carried on by one who is at least slightly familiar with methods of culturing bacteria or fungi. The first

is given as a guide to a rapid method for starting and maintaining the cultures.

1. Prepare nutrient agar slants in test tubes, using the formula given heretofore.
2. Obtain a culture of the amœbæ.[1]
3. Make transplants onto ten tubes.
4. Keep at room temperature out of direct sunlight. Examine after four or five days.
5. Examine in five to eight weeks and make ten new transplants from the best tube. By continuing this method an abundance of amœbæ will be assured at all times.

The second plan of procedure is much more comprehensive and is intended as a guide to those who care to start the method from the beginning.

Isolation of Azotobacter from the Soil

1. Select five grams of poor soil and shake with about thirty cubic centimeters of distilled water.

2. When the soil has settled, inoculate five drops of the liquid into each of ten tubes containing the solution heretofore given, omitting the agar.

3. After three to ten days examine the tubes for Azotobacter. From the tube showing the best growth, inoculate into three tubes containing liquid agar of the given nutrient solution, at 42° C. in the following manner: a. Three loops into the first of the three tubes. Shake well. b. Three loops from the first tube into the second and shake. c. Three loops from the second tube into the third.

(Reference to almost any text book on microbiology will give the general methods for preparing agar slants, and for isolating bacteria by the poured plate method. "Microbiology" by Giltner, Pub. by Wiley and Son is recommended.)

4. Pour the contents of the three tubes into three sterile petrie dishes. Keep the dishes in a cool dark place.

5. After the spot growths have started, examine them for Azotobacter, and make inoculations onto agar slants from the spot

[1]The author will be glad to supply cultures to investigators interested in examining the method. Others may buy them from Mr. W. J. Johnson, 1806 Morse Ave., Chicago, Ill.

showing pure Azotobacter. (If any show soil protozoa, it would be well to make inoculations from them also, as the amœbæ seem to thrive better when associated with them.)

Obtaining Amœbæ

6. At the same time that the work of isolating Azotobacter is being started, take steps to obtain amœbæ. Jenning's (1903: 2406) method of culturing amœbæ and other protozoa for class use is recommended.

7. When the tubes containing Azotobacter are ready, and a culture plentifully supplied with amœbæ is present, inoculate each of the ten tubes of Azotobacter with a few drops from the aqueous culture. This step may have to be repeated a number of times from different cultures until an amœba which will feed on Azotobacter is found.

8. The cultures may be maintained by following steps 1, 3, 4, and 5 of the first method.

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